AN ANALYSIS OF CELL GROWTH AND METABOLISM IN THE CRAYFISH (PROCAMBARUS ALLENI)

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In a recent paper I proposed a general scheme relating cell growth to organism growth with special reference to the problem of body size and metabolic rate (Davison, 1955). Three hypothetical cases were considered. (1) If growth of the animal is exclusively by cell enlargement, cell surface and external body surface each vary directly as the body weight raised to the 2/3 power. (2) If growth is characterized by the progressive formation of cells of fixed size, cell surface will be directly proportional to the body weight raised to the one power. (3) Growth involving the simultaneous addition and enlargement of cells results in cell surface varying as the body weight raised to some power intermediate between 2/3 and 1. In the 1955 paper it was demonstrated that post-metamorphosis growth of frog skeletal muscle is strictly by enlargement of fibers present at the completion of metamorphosis, no new fibers being formed. Both metabolic rate (cc. O_2/hr./gm.) and muscle surface concentration were directly proportional to the body weight raised to the $-1/3$ power.[1] Thus the metabolic rate of the whole animal was directly proportional to the surface concentration of the skeletal muscle cells.

In the frogs studied, the geometric weight increases were small (10- to 50-fold). In further pursuing the problem, I thought it might prove interesting to examine an animal which undergoes a large geometric increase body weight. The crayfish (*Procambarus alleni*) weighs about 4 mg. at the time it becomes independent of the parent crayfish, and increases to an average adult weight of about 16 gm., a 4000-fold increase in body weight. If such growth were isogonic, and the result of cell enlargement, cells would undergo a 13-fold increase in linear dimension ($4000^{\frac{1}{3}}$). Similarly, if the surface law of metabolism were in effect, metabolic rate would undergo a 13-fold decrease over this weight range. Since such dramatic changes in metabolism and cell dimensions seemed unlikely, the crayfish was selected as an interesting object for investigation.

MATERIALS AND METHODS

Animals

Here in the zoology department a breeding population of *P. alleni* has maintained itself in a concrete pool for about 5 years. The pool has a surface area of

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[1] The usual form of the surface law equation is:

$$\text{cc. } O_2/\text{hr.} = k\ W^{\frac{2}{3}}$$

It is more convenient to express metabolism in the form of intensity, thus making it comparable with other information obtained in the form of concentration. Dividing both sides by grams:

$$\text{cc. } O_2/\text{hr./gm.} = k\ W^{-\frac{1}{3}}$$

Similarly, cm.² surface $= k\ W^{\frac{2}{3}}$ becomes:

$$\text{cm.}^2 \text{ surface/cm.}^3 \text{ (gms.)} = k\ W^{-\frac{1}{3}}$$

about 3 m.² and is about 10 cm. in depth. Weak daylight is augmented by continuous illumination from a 300-watt bulb. In addition to the algae which grow in the pool, food is provided in the form of occasional frog carcasses and pellets of mouse food. The population usually consists of 10 to 15 adult animals with a variable number of young depending on the breeding history of the pool. The population can be assumed to be genetically quite homogeneous, since often the surviving adults are siblings from a single successful breeding.

Determination of respiratory rate

P. *alleni* is of the chimney-building variety and is able to survive under moist terrestrial circumstances. Adult animals frequently leave the pond and migrate about the laboratory, especially during rainy weather. It is thus possible to determine the respiratory rate in air with simple volumetric instruments similar to that employed by Flemister and Flemister (1951). These consist simply of two chambers separated by a manometer, one vessel containing the animal and CO_2 absorbent, and the other serving as a compensating vessel. The total volume of the animal chamber can be varied by means of a calibrated syringe, and the gas consumed is measured as the decrease in volume of the animal chamber required to maintain equal pressure in the two vessels. Three volumetric instruments were used. The first two, for larger animals, utilized animal chamber volumes of *ca.* 500 and 50 cc. and oxygen uptakes were measured with 10- and 1-cc. syringes, respectively. The third instrument consisted of two 5-cc. vials connected by a manometer. The volume of the animal chamber was varied by means of a mercury column in a 0.2-cc. calibrated pipette, driven by an ordinary screw clamp. For the smallest animals (4 to 35 mg.) simple capillary respirometers were employed, consisting of vertically mounted capillaries (*ca.* 0.75 mm. bore) attached to horizontal vessels by means of No. 00 rubber stoppers. Vessel volumes were about 0.2 cc. for the smaller and 0.5 cc. for the larger animals. Four instruments, closely matched for final gas volumes and capillary bores, were used in a determination, three being experimental vessels with the fourth serving as a control and thermobarometer. A three-mm. column of kerosene was used as the indicator drop. Results obtained with the capillary instruments agreed well with those obtained with the volumetric instruments on animals of comparable weight. All instruments were carefully calibrated with mercury.

Filter paper, saturated with 10% KOH, was used as CO_2 absorbent, with plastic screening protecting the animal from injury. Experiments in which pure oxygen was flushed through the instrument did not differ significantly from experiments in air. Accordingly, the experiments reported here were performed in atmospheric air. Heistand (1931) reports a T_c of about 40 mm. Hg for oxygen consumption in *Cambarus,* a value well below that approached in the present experiments. The water bath was maintained at 25.1 ± 0.05 C.°

Twenty-four to 48 hours prior to a determination, the animals were isolated in finger bowls or glass aquaria and maintained without food in order to minimize digestive and absorptive activity. After introduction into the respirometer, oxygen consumption was followed with the animal at rest until uptake became linear with respect to time. After the preliminary equilibration of 15 to 30 minutes, most determinations lasted from 60 to 180 minutes. Only one animal was used per

respirometer. The animals were blotted with filter paper and weighed fresh following the determination. No sex differences were observed. Oxygen consumption is expressed as cc. O_2/hr./gm. fresh weight, and gas volumes are reduced to standard temperature and pressure (760 mm. Hg and 0° C.).

No seasonal influence on breeding is apparent in the population, and the metabolism measurements reported here were obtained between September and March.

Determination of cell surface

The animal was weighed, decapitated and the abdomen removed. A small portion of abdominal flexor muscle was dissected out and teased apart on a glass
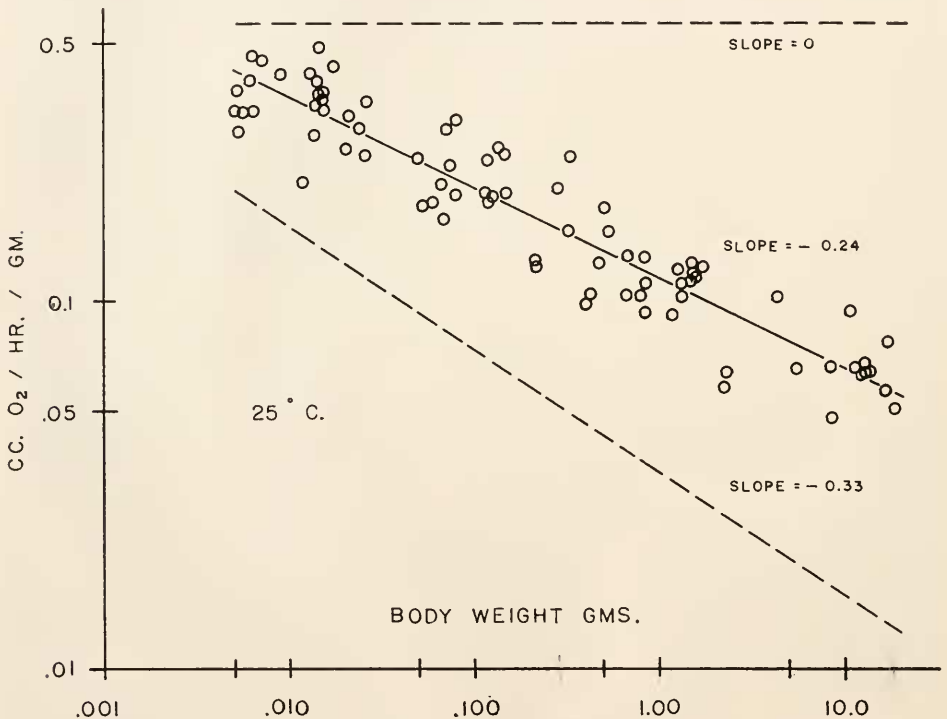

FIGURE 1. Resting oxygen consumption in *Procambarus alleni* plotted against the body weight in grams. Both ordinate and abscissa are in log form. The equation of the experimentally determined line is: cc. O_2/hr./gm. $= 0.115$ (W)$^{-0.24}$. 25° C.

slide. The teased muscle was then stained with 0.75% acetocarmine in 45% acetic acid. It is necessary to stain the muscle cell nuclei in order to easily identify single fibers. The preparation was observed under a suitable power of the compound microscope. About 20 fibers were chosen at random, and the fiber diameters projected on paper by means of the camera lucida. A known linear dimension was projected from a stage micrometer, and the mean fiber diameter determined. For a sample of 20 fibers, the standard deviation was found to be about 20% of the mean. Assuming the fibers approximate long cylinders without free ends, their

surface is given by $2\pi rl$ and their volume by $\pi r^2 l$, so surface per unit volume is the former over the latter or $2/r$ ($4/d$). This quantity, expressed as cm.² surface/cm.³ fibers, is defined as the surface concentration of the muscle. Examination of fiber cross-sections indicates extra-fibral spaces less than 5% of the total area of the section.

Other measurements

Measurements were also made of abdomen length and breadth, eye diameter, and ommatidia facet size. The decapod ommatidium is a receptor unit of a definite
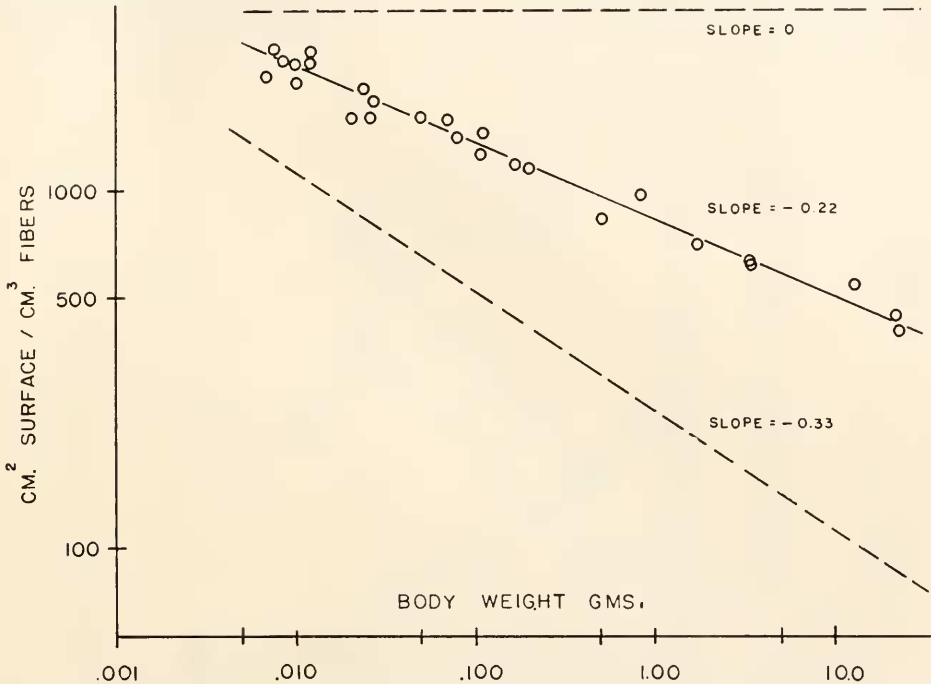

FIGURE 2. Muscle surface concentration plotted against body weight in grams. Both ordinate and abscissa are in log form. The equation of the experimentally determined line is: cm.² surface/cm.³ fibers $= 830 \ (W)^{-0.22}$.

number of cells (Schneider, 1902), so that an analysis of eye size and facet size allows an indirect examination of cell growth and organ growth using the familiar allometric equation of Huxley (1932). Eye diameter was obtained by observing the animal in dorsal aspect and taking [2] the diameter parallel to the longitudinal axis of the animal. Abdomen length was taken as the dorsal distance between the posterior margin of the cephalothorax and the base of the telson. Abdomen breadth is the ventral distance between the pleura of the first abdominal segment.

The size of the corneal facets, which are square in the crayfish, was determined

[2] $y = b \ x^k$. $y =$ linear dimension of organ or cell; $x =$ linear dimension of reference organ or the comparable dimension derived from the body weight ($W^\frac{1}{3}$); $b =$ constant of proportionality; $k =$ allometric coefficient, indicated by the slope when log y is plotted versus log x.

by tracing the outline of a suitable number (20–25) from the most distal part of the eye, again using camera lucida. The most distal facets are about three times as long on a side as those near the base of the eye. The average length on a side of the distal facets was recorded together with the data on eye diameter, abdomen length and breadth, and the body weight in grams.

<div align="center">RESULTS</div>

Respiratory rate

In Figure 1 is plotted resting metabolic rate versus body weight in grams, each scale being in logarithmic form. The straight line, drawn by eye, has a slope of
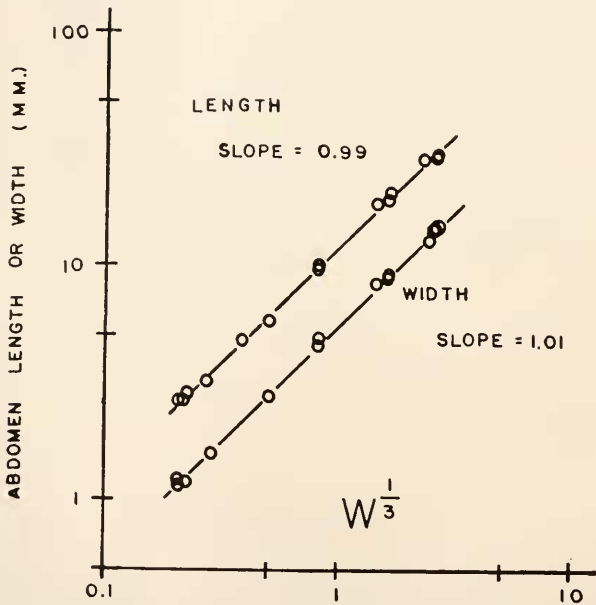

FIGURE 3. Abdomen length (upper plot) and abdomen width (lower plot) plotted against the cube root of the body weight in grams. Both coordinates are in log form. The equations of the lines are as follows: Abd. length (mm.) = 12 $(W^{\frac{1}{3}})^{0.99}$. Abd. width (mm.) = 5.5 $(W^{\frac{1}{3}})^{1.01}$.

−0.24, a value intermediate between 0 (metabolic rate independent of body weight) and −0.33 (metabolic rate following the surface law). Metabolic rate decreases from about 0.355 cc./hr./gm. at 10 mg. to 0.066 cc./hr./gm. at 10 gm., about a 5.4-fold decrease in rate, and considerably less than the 10-fold decrease expected on the basis of the surface law. Weymouth *et al.* (1944) reported a similar value for the exponent (−0.20) in a study of the metabolism of *Pugettia* and other marine crustacea. In *Procambarus,* the rate at 10 gm. (0.066 cc./hr./gm.) compares favorably with the respiratory rate reported for the European lake crayfish *Astacus leptodactylus* (0.07 cc./hr./gm. at 20 C.°) (Prosser *et al.,* 1950).

Muscle cell surface

Figure 2 represents the calculated surface concentrations obtained from average fiber diameters plotted against body weight in grams, again in double logarithmic form.   The straight line, fitted by eye, indicates a slope of −0.22, indicating that growth of the muscle is accomplished by the enlargement of fibers, once formed, and the progressive addition of fibers throughout the weight range.   By exploring the teased and stained preparations, it is possible to observe stages in fiber formation. Spindle-shaped myoblasts come to lie together in closely packed longitudinal groups, with subsequent loss of their cell boundaries, and a peripheral arrangement of the
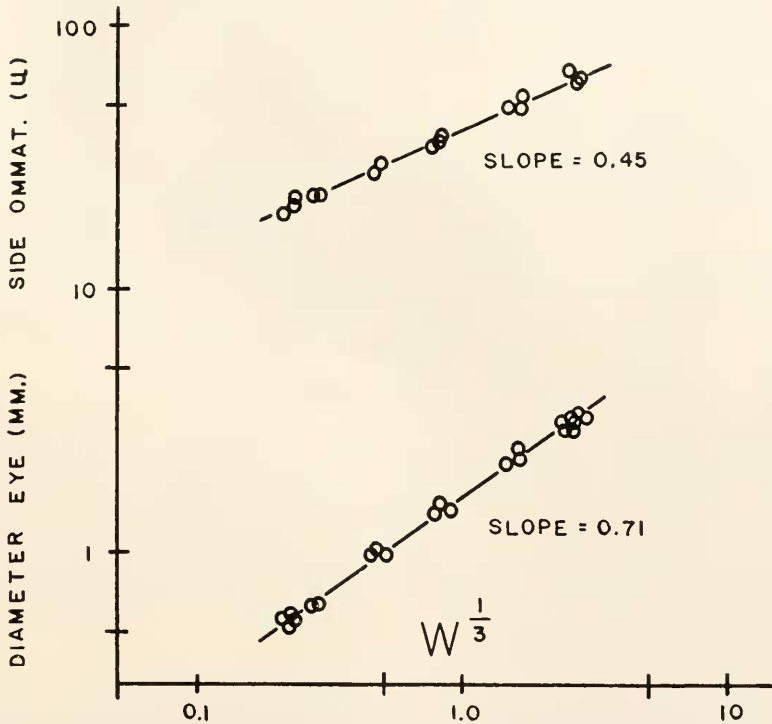

FIGURE 4.   Eye diameter and ommatidia facet length plotted against the cube root of the body weight in grams.   Both coordinates are in log form.   The equations of the lines are as follows: side ommatidium $(\mu) = 39.8$ $(W^{\frac{1}{3}})^{0.45}$, diameter eye (mm.) $= 1.62$ $(W^{\frac{1}{3}})^{0.71}$.

fiber nuclei.   (For a discussion of the histogenesis of decapod skeletal muscle see Schneider, 1902.)   It is not clear from the present study at exactly which stage in fiber growth the myoblasts stop contributing to the fiber.   However, the spacing of the nuclei is much greater in large than in small fibers, indicating that the individual fiber receives all its nuclei while still relatively small.

Surface concentrations decrease from about 2240 cm.²/cm.³ at 10 mg. to 500 cm.²/cm.³ at 10 gm., corresponding to about a 4.5-fold increase in fiber diameters. The lower dotted line in Figure 2 indicates the 10-fold decrease which would occur if the fibers grew entirely by enlargement.

The observed 4.5-fold increase in fiber dimensions allows an indirect calculation of the increase in fiber numbers for a 1000-fold increase in weight. It may be assumed that fiber lengths are determined by the anatomical features of the origins and insertions, and accordingly undergo a 10-fold increase between 10 mg. and 10 gm. Since fiber diameters would also increase by a factor of 10 if their number remained constant, the relative increase in number of fibers is given by $(10/4.5)^2$ or 4.9. Thus, a weight increase of 1000-fold is accompanied by about a 5-fold increase in fiber number.

An alternative possibility to the addition of fibers during growth is that the abdomen might be markedly heterogonic with respect to the body as a whole. In
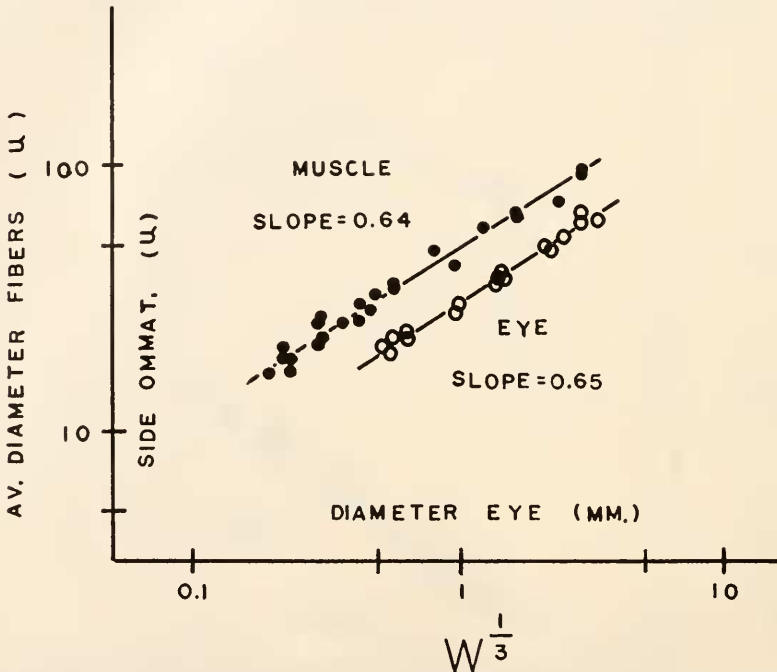

FIGURE 5. Average diameter muscle fibers plotted against the cube root of the body weight in grams (upper plot), and ommatidia facet length plotted against the diameter of the eye (lower plot). All coordinates are in log form. The equations of the lines are as follows: Av. fiber diam. $(\mu) = 49 \ (W^{\frac{1}{3}})^{0.64}$; side ommatidium $(\mu) = 29.5 \ (\text{diam. eye (mm.)})^{0.65}$.

order to test this possibility, abdomen length and breadth have been plotted against the dimensionally comparable quantity derived from the body weight (cube root of weight). The double log plot in Figure 3 indicates slopes for both length and breadth very close to unity, being 0.99 and 1.01, respectively. In other words, the relationship between fiber dimensions and body size is essentially identical to that between fiber dimensions and abdomen size. The abdomen and the whole body are isogonic.

Ommatidia and eye growth

The eye and its ommatidia are especially interesting in this connection, since each is markedly heterogonic with respect to body weight. Figure 4 illustrates

the allometric relationship between eye diameter (mm.) and ommatidia facet length (μ) when each is plotted against $W^{\frac{1}{3}}$. The slopes for each are less than unity, 0.71 for the eye, and 0.45 for facet length. The lower value for facet length indicates that growth of the eye is characterized by the progressive addition of receptor units. Knowing the allometric coefficient (slope) for each with respect to $W^{\frac{1}{3}}$, it is possible to calculate the allometric coefficient of ommatidia size with respect to eye size. This will be the ratio of the slopes or 0.45/0.71 which equals 0.635.[3]

Since average muscle fiber diameters increase systematically with body weight, it should also be possible to express this growth according to Huxley's allometric formulation. In Figure 5 are plotted both average fiber diameter (μ) against W (upper plot) and, on the basis of direct measurements, facet length (μ) against eye diameter (mm.). Fiber diameter indicates a slope of 0.64 and facet length a slope of 0.65, each value agreeing closely with the calculated slope (0.635) for facet length versus eye diameter obtained above.

Since the eye is a two-dimensional (surface) system of receptor units, the relationship between number of ommatidia and size of the eye will be essentially identical with that between number of fibers and size of the abdomen, in which the increase in number is also dependent on two-dimensional quantities (cross-sectional area of the abdomen and the fibers).

The close agreement of the allometric coefficients for the ommatidia-eye system and the fiber-body system indicates that the same basic mechanism of simultaneous addition and enlargement operates in each.

DISCUSSION

It is clear, then, that a departure from the surface law of metabolism has been associated with a quantitatively similar departure in the manner of cell growth. Both metabolic rate and muscle surface concentration vary as the body weight raised to a power intermediate between -0.33 and 0. These observations are consistent with the view that the metabolic rate of the whole animal may be determined by the cell surface of the skeletal muscle. In addition, another organ system, the eye, indicates a manner of growth which is quantitatively comparable to that of the body as a whole.

Huxley (1932) has compiled a list of arthropod allometric coefficients drawn largely from Teissier's paper (Teissier, 1931). In the European walking stick (*Dixippus morosus*) values of the allometric coefficient are 0.48 and 0.37 for the eye and ommatidia diameters, respectively (measured against total length), giving a value of 0.77 (0.37/0.48) for ommatidia diameter versus eye diameter, and indicating a manner of growth comparable to that observed in the present study. Trager's analysis indicates that the tissues of the flesh fly larva (*Lucilia sericata*) increase in size solely as the result of cell enlargement (Trager, 1935). He also cites a number of earlier papers suggesting the growth of larval tissues by cell enlargement. Among these is a study by August Weismann (1864) who noted an

[3] log (facet length) $= 0.45$ log $(W^{\frac{1}{3}}) +$ log b, and: log (eye diameter) $= 0.71$ log $(W^{\frac{1}{3}}) +$ log b'. Solving each for log $(W^{\frac{1}{3}})$: $\dfrac{\log\ (\text{facet length})}{0.45} -$ log b $=$ log $(W^{\frac{1}{3}})$ $=\dfrac{\log\ (\text{eye diameter})}{0.71} -$ log b', and eliminating the common term: log (facet length) $= 0.45/0.71$ log (eye diameter) $+$ log b $-$ log b'.

increase in size but not in number of muscle cell nuclei during the larval growth of muscid flies.

I would like to point out that the findings of the present study, as well as those cited by reference, are not in agreement wth Zeuthen's repeated statements to the effect that metazoan growth is characterized by an increase in number and not in size of cells (Zeuthen, 1953, 1955).

However, Zeuthen has made what may be a very pertinent observation when considered from the point of view developed here. Considering the animal kingdom as a whole, he points out that both in very small (less than a few mg.) and in very large (several kg.) animals, metabolic rate tends to change less markedly with body weight than in animals from the middle range of weight (Zeuthen, 1949). A possible explanation for this phenomenon may reside in a consideration of cellular structure. In very small animals a practical lower limit in cell dimensions may be reached so that smaller animals are composed of fewer rather than smaller cells. Similarly, there may also exist an upper limit on cell dimensions beyond which larger animals would be composed of more rather than proportionately larger cells.

Bertalanffy and co-workers (1951, 1953) prefer to attribute the phenomenon of size-dependent metabolism to "organismic factors." Since the phenomenon is expressed by the intact organism, there seems little doubt that organismic factors will play their part. However, cellular structure is not thereby precluded as a determining or, under standard conditions, a limiting factor in the expression of metabolism by the whole animal. The argument developed here points to a view of the organism as a highly integrated cell aggregate or collection of unit organisms (cells) whose growth, and hence structural, properties determine the performance of the animal proper. Virchow expressed a similar, if oversimplified, view of the organism in 1858 when he said, "Every animal appears as a sum of vital units, each of which bears in itself the complete characteristics of life." [4]

Summary

1. The resting metabolism of *Procambarus alleni* was determined over about a 4000-fold weight range. Metabolic rate was found to vary directly as the body weight raised to the − 0.24 power.

2. Abdominal muscle fiber diameters were determined over about a 4000-fold weight range. Calculated surface concentration was found to vary directly as the body weight raised to the − 0.22 power, providing evidence that the growth of the skeletal muscle is characterized by simultaneous addition and enlargement of fibers throughout the weight range.

3. Allometric analyses were made of fiber diameter, eye diameter, and ommatidia size with respect to the cube root of the body weight. The analyses indicated that the growth of the eye with respect to its constituent units is quantitatively similar to the growth of the abdomen with respect to its skeletal muscle cells.

4. The close similarity between the allometric coefficients of metabolic rate and surface concentration was taken as evidence that the metabolic rate of the whole animal may be dependent on the cell surface of the skeletal muscle.

[4] Quoted in introductory chapter to E. B. Wilson, The cell in development and heredity, 1925.

5. Evidence obtained from the literature was cited for other examples of the growth of arthropod tissues by cell enlargement.

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